Protein Crystallization Apparatus for Microgravity

Daniel C. Carter/ES01
205–544–5492
E-mail: Dan.Carter@msfc.nasa.gov

The major objectives of this research are aimed at the design, construction, and operation of an improved microgravity protein crystal growth hardware and facility.

Previously, in response to the first NASA biotechnology NRA, we proposed to develop and construct a facility based protein crystal growth concept. This was undertaken largely because of the many important examples of quality improvements gained from growth in the diffusion-limited environment in space. The concept was based on the adaptation for microgravity of a commonly used disposable crystallization tray thereby improving sample logistics and handling, as well as greatly increasing the potential coinvestigator group and science return. The proposal proceeded in two phases, in the first stage a hand-held concept was to be developed and tested in microgravity to test the principal/concept. If successful, we further proposed to construct a facility for the mass production of protein crystals for application in the structure determination. Access to the facility is open to scientist from industry, academia and government laboratories. The principal aims of the research are:

- Establish a protein crystal growth facility which would provide greatly increased experiment and coinvestigator capacity in order to increase the odds of obtaining crystals of improved quality for application in this atomic structure determination, consequently increasing the overall science return from each mission;
- Investigate the disposable interface concept in development of microgravity hardware for reduced cost and improvements in logistics and handling; and
- Utilize the facility to delineate factors contributing to the effect of microgravity on the growth and quality of protein crystals.

Improvements in the quality protein crystals can be the limiting step in the determination of the atomic structure. A detailed knowledge of the atomic three-dimensional structure of protein molecules is key to a fundamental understanding of biochemistry and is of tremendous application in structure-based drug design. Further, examination of crystals grown in microgravity provides important scientific data which illuminates the mechanisms involved in the growth of high-quality crystals in the microgravity environment.

During the course of this investigation hardware design and construction has proceeded rapidly through hand-held versions to complete facility hardware. A large flight coinvestigator group has been established and the hardware has been manifested and flown succesfully on several Shuttle missions (table 10). Additionally, special dialysis counter-diffusion hardware has been designed, constructed and is now currently active on Mir. The accomplishments from both the hardware development and the results from the flight experiments have been outstanding. Over the course of 3 flights of the protein crystallization apparatus for microgravity (PCAM) facility hardware in 1995, over 1,600 individual vapor equilibration experiments were flown, exceeding the total number flown in under the Microgravity Science and Applications Division (MSAD) program since the inception of the protein crystal growth program in 1985. It should be noted that the PCAM hardware pioneered the development and deployment of disposable interface in flight hardware which has resulted in greatly reduced costs, as well as numerous additional advantages. Highlights of the PCAM and diffusion-controlled crystallization apparatus for microgravity (DCAM) experiments include crystals of human antithrombin III (Dr. Mark Wardell, Washington University) which were of greatly improved quality. These crystals have allowed for the completion of the atomic model of this important protein. Improved crystals have also been grown of neurophysin/vaspressin complex, liver augmenter, (Dr. B.C. Wang, Dr. John Rose, University of Georgia) raf kinase, (Drs. J.P. Wery and David Clawson, Eli Lilly) and the nucleosome core particle (Dr. Gerry Bunick and Joel Harp, Oak Ridge National Laboratory). Several publications describing the improvements gained from microgravity are in preparation. 1996 marked the first long-duration experiment for DCAM. Crystals produced from DCAM returned from STS–79 after a 6-month stay on Mir were recently distributed to the guest investigator group. Notable accomplishments include numerous large, spectacular crystals of the nucleosome core particle which were the largest grown to date (Dr. Gerry Bunick and Joel Harp, Oak Ridge National Laboratory) and the largest crystal ever produced of T7 RNA polymerase (Dr. B.C. Wang, Dr. John Rose, University of Georgia). A summary for the

TABLE 10.—*Flight hardware characteristics.*

Mission	Hardware	# Sample Chambers	# Investigators	# Proteins
STS–62	HH-PCAM	96	1	4
STS–63	6 PCAM	378	7 (5 groups)	9
STS–67	6 PCAM	378	11 (8 groups)	9
STS–73	12 PCAM	756	13 (8 groups)	12
	8 Short PCAM	168	1	
	81 DCAM	81	3	3
STS–76	162 DCAM	162	8 (7 groups)	9
STS–79	162 DCAM	162	9 (7 groups)	11

flight experiments conducted to date are synoposized in table 10. Several publications describing the effects and improvements gained in microgravity are in preparation.

Carter, D.: "PCAM and DCAM, Summary of Flight Results 1994–96." Protein Crystal Growth Workshop, Panama City, FL, May 1996.

Carter, D.: "PCAM and DCAM, Summary of Flight Results 1995–96." Gordon Research Conference, New Hampshire, June 1996.

Carter, D.: "PCAM and DCAM Hardware and Facility," International Microgravity Protein Crystal Growth Workshop, University of California, Riverside, 1996.

Sponsor: Microgravity Science and Applications Division, NASA Headquarters

University/Industry Involvement: Eli Lilly and Company; Monsanto/Searle; DuPont Merk; Oak Ridge National Laboratory; Washington University School of Medicine, St. Louis; University of Louvain, Belgium; University of Georgia; University of Pittsburgh; Virginia Commonwealth University; University of Saskatchewan, Canada; University of Colorado; University of Groningen, The Netherlands; Justus-Liebig University, Giessen, Germany; University of Helsinki, Finland; Max-Delbruck-Centrum für Molekulare Medizin PG Kristallographie, Berlin, Germany; Institut de Biologie Structurale Jean Pierre Ebel, Grenoble, France; Rosenstiel Basic Medical Sciences Research Center at Brandeis University, Maryland.

Biographical Sketch: Dr. Dan Carter is the senior scientist for biophysics at Marshall and is director of the Laboratory for Structural Biology. His accomplishments include the crystallization and atomic structure determination of several important proteins including serum plasma albumin. His research has been published in scientific journals, featured on the cover of *Science*, and he has been selected for NASA Inventor of the Year awards. His current research interests in structural biology include important HIV proteins, improvements in drug efficacy and delivery through rational drug design, and blood replacement products. Carter received his Ph.D. in x-ray crystallography from the University of Pittsburgh in 1984.